INSTABILITY OF THE MATING TYPE ALLELES IN SACCHAROMYCES[1]

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COMPLETE TRANSFORMATION OF SACCHAROMYCES INTO TORULAE

Copulation in *Saccharomyces cerevisiae* is controlled by a pair of *a*/*α* alleles. Ascospores are haploid for either the *a* or the *α* gene and produce clones, the members of which copulate pairwise when the cultures are mixed. The *a* by *α* matings result in abundant copulations, forming legitimate diploid zygotes capable of undergoing reduction to produce viable 4-spored asci. The *a* by *a* and the *α* by *α* matings fail to result in copulations. The four cultures from the single haploid ascospores which were the basis of the experiments and illustrations in our earlier papers (Lindegren and Lindegren, '43 b, c) have been carried in culture in the laboratory for over a year and have been observed to produce a great variety of morphological mutants. That they still retain their haploid character is shown by the shape and size of the cells. Recently the earlier experiments were duplicated by mating the cultures in all possible pairs, following exactly the same procedure as before. These matings failed to produce either copulations or diploid cells. No asci were obtained by transferring the mated cultures to our presporulation medium and gypsum slants (Lindegren and Lindegren, '44). The cultures are now completely neutralized as to sex and have been transformed into typical members of the genus *Torula* or *Torulopsis*. They are incapable of producing diploid sporulating cultures either by legitimate or illegitimate copulation. This is undoubtedly due to mutation either of the principal *a*/*α* alleles or of modifying genes which inhibit copulation.

The genus *Torula* or *Torulopsis* was invalidated by Šatava ('34) and Winge and Laustsen ('39) suggested that the genus *Zygosaccharomyces* should be dropped. We have presented evidence on both these points ('43b), and the names of the genera are used in the present discussion without implication of generic status.

THE EFFECT OF CAMPHOR ON COPULATION

Thaysen and Morris ('44) produced large, presumably diploid cells of *Torula utilis* by plating the organisms on beer wort agar containing 0.3 per cent camphor. We tested the effect of camphor on a number of yeast cultures and found that with concentrations of 0.3 per cent camphor about half of the cultures produced outgrowths strongly resembling copulation tubes; and with 0.5 per cent camphor a much larger proportion of cultures produced an abundance of these structures.

[1] This work was supported by a grant from Anheuser-Busch, Inc., St. Louis.

Plate 7 shows the effect of 0.3 per cent camphor on a diploid and a haploid culture.

The fact that camphor stimulates the production of structures resembling copulation tubes suggested that Thaysen and Morris had been able to produce a large *Torula* by inducing copulation between normally stable haploid cells and led to the following experiment. The four single ascospore cultures, referred to above, which had become transformed into *Torulae*, were mated in all combinations in malt-dextrose-yeast extract broth containing 0.5 per cent camphor and compared with a control in which matings were made in the absence of camphor. In the presence of camphor many tubes grew out and anastomoses were visible between adjacent cells. Cells from each copulating culture were transferred to our presporulation medium and then to gypsum slants. No diploid cells or spores were obtained as a result of the above matings, either with or without camphor, indicating that in spite of the abundant production of tubes none of the cell fusions had been followed by nuclear fusion.

This first failure to effect nuclear fusion by the use of camphor was followed by a second experiment with fresher cultures. Four cultures, which had been obtained from four single ascospores of a single ascus about six months before, were similarly mated in the presence and absence of camphor. The serial numbers of these four cultures are 61, 62, 63, and 64. Cultures 61 and 62 are typically round-celled, while 63 and 64 are somewhat ellipsoidal. Earlier tests had shown that 61 and 62 were of the same mating type, while 63 and 64 were of the opposite. Outline drawings of the cells mated in broth without camphor are shown in fig. 1. Originally the cells of 63 and 64 were about the same size but some of those of culture 63 are seen to be much larger than the standard haploid ones and are characteristically diploid, indicating that this culture had become diploid spontaneously by illegitimate copulation. This culture, therefore, contains a mixture of haploid and diploid cells.

The results of the pairings shown in fig. 1 indicate that copulations failed to occur when cultures 61 and 62 were mated, and also with 63 and 64, but copulations occurred in the other four possible combinations. This proves that the four cultures still retain the same mating type differences which had characterized them in the previous experiments. It is probable that only the haploid cells in culture 63 mated, but it would require further analysis to determine this specifically.

Cells from the culture tubes in which the matings were made were transferred to our presporulation medium and, after the proper interval, were placed on gypsum slants. The photographs in pl. 8 show the ascospores obtained on gypsum slants, and confirm the findings of the previous matings. Cultures 61, 62, and 64 produced no ascospores; culture 63, however, which had contained illegitimate diploids, developed a few ascospores. Many of these were characteristically aborted and most of them were probably non-viable. This culture is unusual for an illegitimate diploid inasmuch as it produces a relatively large

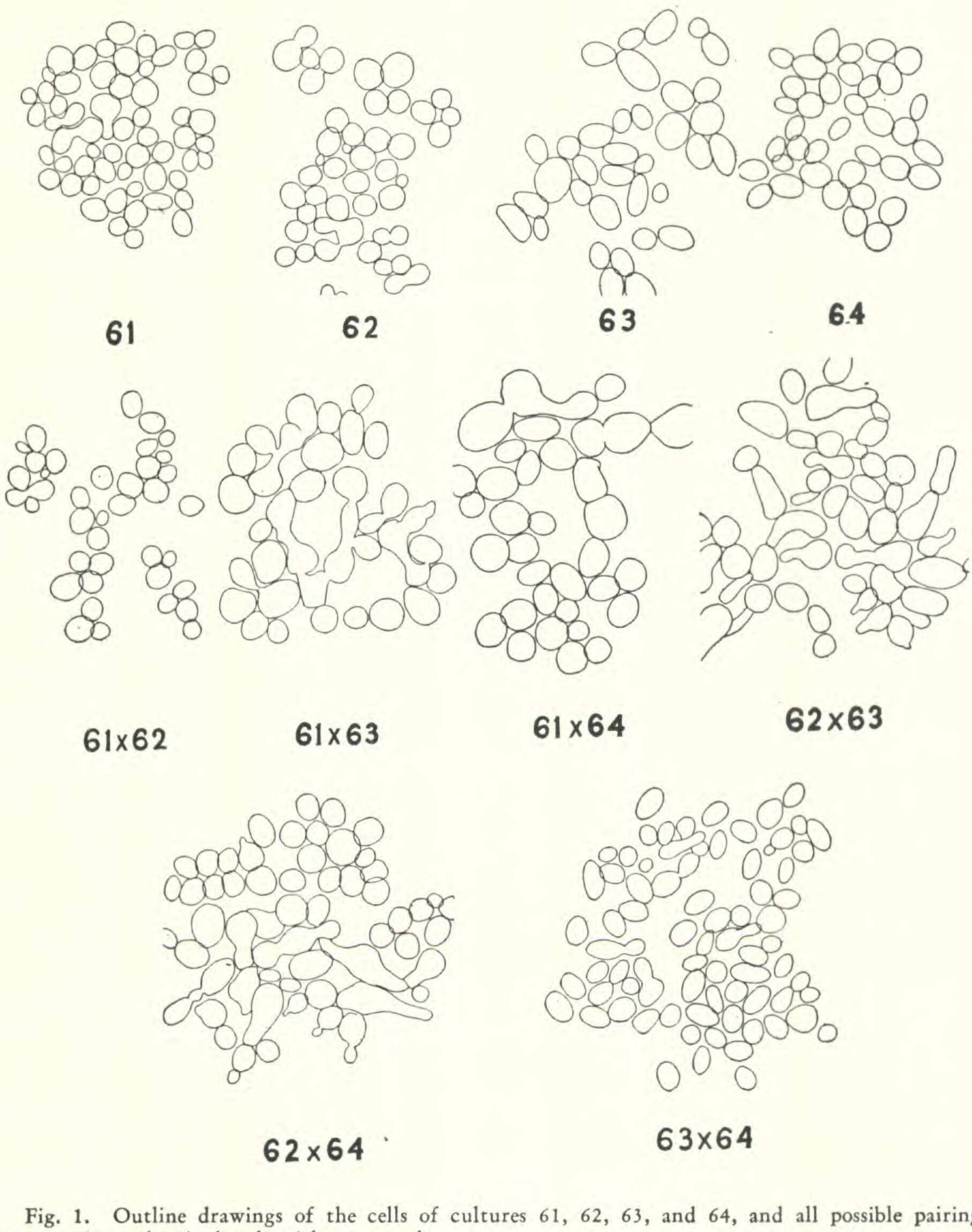

Fig. 1. Outline drawings of the cells of cultures 61, 62, 63, and 64, and all possible pairings from mating tubes in broth without camphor.

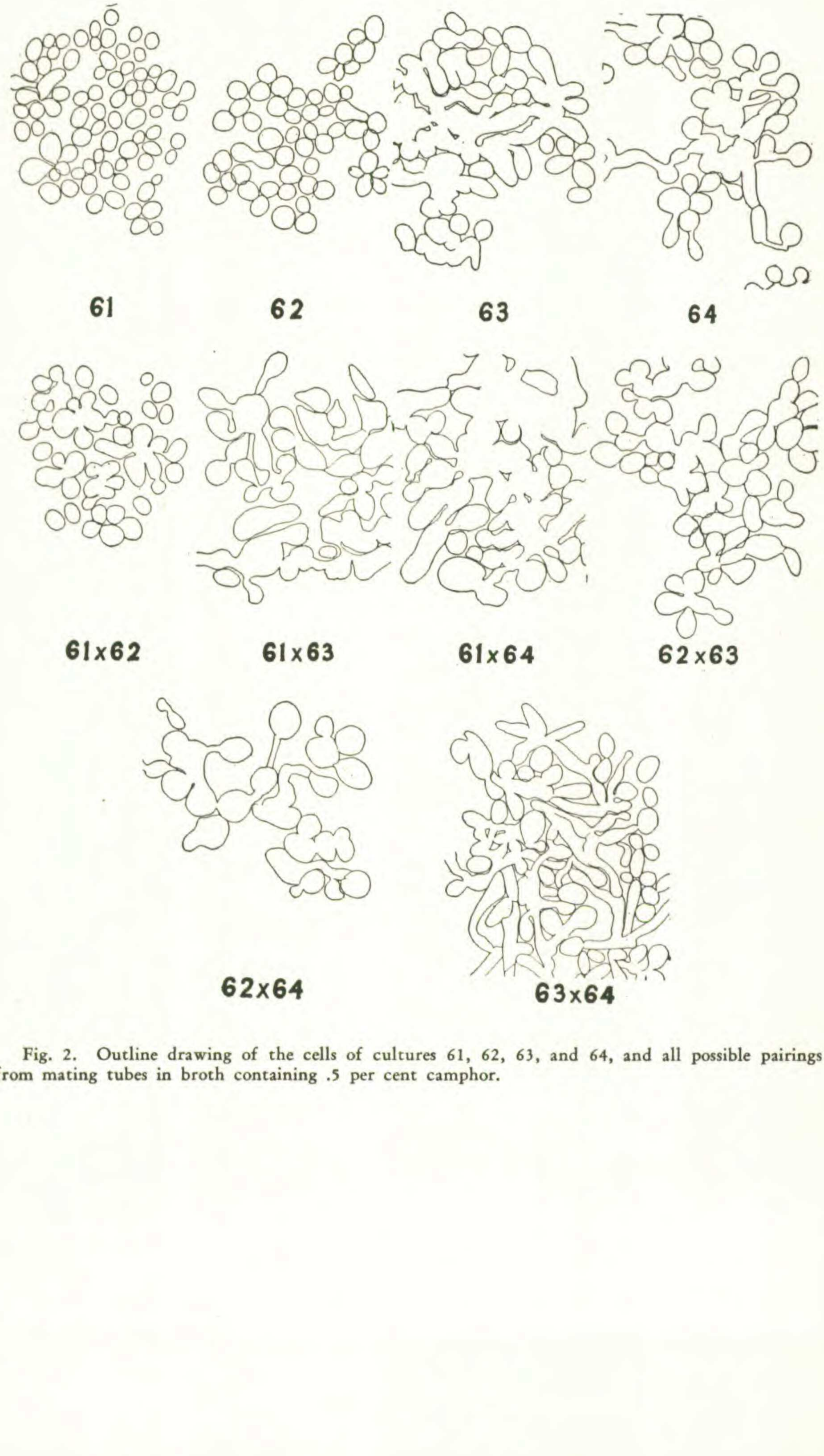

Fig. 2. Outline drawing of the cells of cultures 61, 62, 63, and 64, and all possible pairings from mating tubes in broth containing .5 per cent camphor.

number of 4-spored asci. The viability of the ascospores from cultures of this type is generally quite low, and the production of ascospores in culture 63 was also much lower than that of the four legitimate diploids.[1] Mating of 61 by 62 did not result in the production of ascospores, and fig. 1 shows that the mating had not resulted in cell fusion. The mating of 63 and 64 did not produce any more ascospores than 63 alone. Mating 62 by 64 resulted in the production of many asci only a few of which were 4-spored. The other three legitimate matings gave relatively high frequencies of 4-spored asci, with 62 by 63 conspicuously better than any of the others.

A duplicate experiment was carried out, using precisely the same methods except that 0.5 per cent camphor was added to the medium in which the matings were made. The outline drawings of the cells from the matings shown in fig. 2 indicate that cultures 61 and 62 were relatively insensitive to the effects of camphor, while in cultures 63 and 64 anastomoses occurred frequently. In the mating of 61 by 62, a few of the clusters are swollen, but the indications are generally consistent with the low sensitivity of these cultures to camphor. A mixture of 63 by 64 in the presence of camphor resulted in an excessive number of anastomoses. After growing these cultures on presporulation media, they were transferred to gypsum with practically the same results as obtained without the use of camphor. In the mating 62 by 64, 4-spored asci were very rare; the best 4-spored asci were produced by mating 62 by 63. The results of both matings were the same as in the absence of camphor. In spite of the abundance of anastomoses in the 63 by 64 mating, no more spores were produced by the combination than by 63 alone.

These results indicate that, except for structures similar to copulation tubes, matings in the presence of camphor were not able to effect combinations which did not occur in its absence. This does not prove that camphor is ineffective; it may be that, combined with other substances present in the type of beer wort used by Thaysen and Morris, copulations could be induced which would result in nuclear fusion. The importance of the subject warrants extensive examination of the phenomenon. Critical control experiments are required to exclude the alternative that the diploidization may have occurred spontaneously.

THE INSTABILITY OF THE MATING TYPES UNDER SELECTION PRESSURE

The transformation of the yeast cultures capable of copulation into neutral *Torulae* explains the fact that many of the cultures from single ascospores which we carried in culture for a year or more were unable to copulate with freshly isolated tester strains capable of copulation. Apparently, the genes controlling copulation become unrecognizable by mutation under the selection pressure exerted on haploid cultures. These observations also explain the results

[1] The fields from culture 63, selected for the photographs, contained more than the average number of ascospores, but in photographing the other cultures, an attempt was made to select representative fields.

obtained by Winge and Laustsen ('37), who paired all the available *Torulae* in all combinations and failed to obtain any diploid cells. When cultures are isolated in the haploid state, the survival value of the genes controlling copulation disappears and a new mutation, even if its advantage be very slight, would tend to replace either the *a* or the *α* genotype. It is not required that the new mutation appear at the *a* or *α* locus. It may be a modifier or *a* or *α* with some slight advantage over its parent type.

A second type of transformation with respect to copulation capability is found rather infrequently among single ascospore cultures. These variants produce an abundance of copulation tubes as soon as the culture reaches full growth. The cells from these cultures can copulate with the cells of any other culture capable of copulation and may produce diploid zygotes, irrespective of whether or not they are mated with either *a* or *α* mating types. The variants have lost the characteristic of discriminating between the *a* and *α* types. This non-discriminatory type may have been produced by a mechanism similar to that suggested by Sturtevant ('24) to explain orthogenesis. The gene producing copulation tubes may have either mutated to a gene which retains the quality of producing copulation tubes associated with another character of high survival value or may be closely linked to a second gene of high survival value. To quote Sturtevant: "If we suppose that the antlers of the Irish elk were dependent for their size upon testicular secretions, then selection may have increased the testicular secretions for reproductive or other reasons, and thus have resulted in a purely incidental increase in size of antlers." These haploid yeasts have been transformed into the "genus" generally recognized as *Zygosaccharomyces*.

The instability of genes controlling copulation in yeasts is paralleled by a similar instability at the *AB* loci in the *Hymenomycetes*. Two cultures of *Coprinus lagopus* collected from different dung heaps are completely interfertile (Hanna, '25) due to mutations at the *AB* loci. Vandendries ('37) extended this study to show that sterility factors can be demonstrated when *Hymenomycetes* from different continents are mated. The self-sterility alleles in *Neurospora* are extraordinarily stable (Lindegren, '32, '34), and of many single ascospore cultures examined we found no mutations at the +/— locus. Single ascospore cultures of *Neurospora* often lose their fertility when carried in culture; this is probably due to genes modifying the +/— factors. The fact that multiple mutants are less fertile than the wild type suggests that most mutant genes modify the +/— alleles to reduce fertility (Lindegren, Beanfield and Barber, 1939).

Haploid yeasts are astonishingly mutable. Subculturing a haplophase yeast produces a large variety of morphological mutants. If the *a*/*α* alleles are modified by these mutant genes, changes in fertility might occur. This would be especially true in a yeast culture, since the selection pressure is great and constantly varying, due to the effect of the growth of the organisms on the substrate. Diploid cultures are protected against variation by the presence of the normal allele at each locus to "cover" the mutant gene which is generally recessive. Homozygous

diploids are the most stable of all since they generally fail to produce viable haploid ascospores, and the diploid ascospores which they produce germinate to form clones indistinguishable from the parent type. A parallel stability with regard to mating type is found in completely homozygous diploid cultures of *Paramecium* (Sonneborn, personal communication) which are practically non-variable in their sex reactions.

Since haploid yeast cultures are sexually unstable, when subcultured vigorously the test for mating type can only be performed with single-ascospore cultures obtained from freshly isolated spores.

CRITERIA FOR DISTINGUISHING HAPLOID FROM DIPLOID CULTURES

Cell size alone is not a sufficient criterion for distinguishing diploids from haploids because cells in both types of cultures vary in size. Neither is cell shape in itself a satisfactory criterion of haploidy, for while the cells of haploid cultures are generally round many are distinctly ellipsoidal. Haploid cultures are usually more variable than diploid cultures, and old cultures may contain larger cells than do diploid ones. Comparative measurements of cell size must be made on young cultures grown under standard conditions in liquid medium. An example of the bloated, round cells frequently found in haploid cultures is seen in pl. 7c. Such cells invariably have an enlarged central vacuole, suggesting that increase in size is not due to any additional dry matter. Cells of this type, often much larger, are found in old cultures and can easily be distinguished from normal ellipsoidal, diploid cells. Extremely thin, elongated cells are also found in haploid cultures. Much of this variation is simply phenotypic, as is indicated by the fact that cells from different parts of the colony vary in size and shape (Lindegren and Hamilton, '44). Yeasts cultured on agar show a pseudomycelium of elongated cells growing into the substrate. The cells on the top of a colony of *Torula utilis* are often extremely elongated and much smaller than those found in liquid medium. Both bloated cells and long, thin cells are generally characteristic of haploid cultures, although many haploid cultures show a predominance of one or the other type. They are generally absent from diploid cultures.

Another fairly constant distinction between haploid and diploid cultures is that haploid cells often tend to form associations due to failure of the daughter cells to separate after budding. When only four cells are present in a cluster, the typical figure-8 configuration described by Winge ('35) results. These clusters may become rather large and are generally characteristic for specific mutant types. In forming clusters, the first bud does not always persist with its long axis perpendicular to the tangent of the cell surface. The persistent perpendicular bud is characteristic of diploid cultures.

A typical diploid cell produces only a single bud at a time. However, many haploid cultures contain single, large, round cells which produce many buds at the periphery. This type of budding has been used as a generic criterion in the

imperfect yeasts, but it is found not infrequently in single ascospore cultures isolated from the standard *Saccharomyces cerevisiae*.

Many haploid cultures contain pear-shaped cells resembling the first stage of copulation tube formation; in unmated haploid cultures, the stimulation required to complete the tube is not present. Pear-shaped cells are characteristic of apiculate yeasts.

Diploid cultures therefore differ characteristically from haploids in the following manner: (1) they generally fail to remain associated after the bud has attained full size; (2) only a single bud, generally at right angles to the cell surface, is formed; (3) they are generally more uniform in cell-size than haploid cultures; (4) there are practically no elongated, balloon or pear-shaped cells, while these forms abound in haploid cultures, especially in old ones.

In addition to a greater stability in cell-size and shape, diploid colonies are remarkable in producing uniform, large, smooth colonies, while haploid cultures produce a great variety of small, rough colonies (Lindegren and Lindegren, '43a).

CRITERIA FOR ESTABLISHING THE OCCURRENCE OF NUCLEAR FUSION IN YEASTS

When anastomoses are found in yeast culture, it is difficult to see whether or not true fusions have occurred. Cell fusions in the fungi are often without any sexual significance and are not necessarily followed by nuclear fusions. In different fungi, different criteria are relied upon to prove that copulation and nuclear fusion have occurred. In the *Hymenomycetes*, clamp connections are evidence of copulation but do not necessarily indicate that nuclear fusion will follow. In *Neurospora*, the development of perithecia and ascospores in mixed cultures proves that mixing the cultures has resulted in nuclear fusion. In the yeasts, copulation between the cells of mixed cultures followed by the production of the typical diploid cells is suggestive, but since copulation and diploid cells often occur spontaneously in haploid cultures diploid cells are not necessarily evidence that a hybrid has been formed. The occurrence of anastomoses in old haploid cultures at the center of the cell aggregations is in line with Winge's observations that the figure-8 conformation so frequently found in haploid yeasts is a preliminary to illegitimate copulation.

Nickerson and Thimann ('43) stated that when two cells of a *Zygosaccharomyces* are attached, budding can be distinguishel from copulation by the fact that buds always occur with their long axis at right angles to the tangent of the cell surface. This is generally true of diploid cultures (see above) but they were working primarily with *Zygosaccharomyces*, which are haploid. In haploid cultures the clover-leaf cell aggregates are often formed by two secondary buds appearing near the point of attachment of the budded cells. There are indications of post-fission movement at the attachment point, which results in a change of the perpendicular attachment of the bud. The only conclusive proof that hybridization between the mixed cultures has occurred is the subsequent production of

large diploid cells capable of undergoing meiosis and producing viable 4-spored asci.

BIOLOGICAL IMPLICATIONS OF THE INSTABILITY OF THE MATING TYPE ALLELES

In nature a *Saccharomyces* which yields a large number of vigorous *Torulae* will be at a disadvantage, for the *Torulae* might displace the parent form. The *Torulae* are a fundamentally less efficient biotype, since they are incapable of exploiting the sexual mechanism for effecting genic recombinations. In *Saccharomycodes*, all copulations occur in the ascus and no haplophase is found. Suppression of the haplophase in *Saccharomycodes* does not permit selective competition between gametes which occurs in *Saccharomyces* just previous to copulation. This competitive interphase eliminates the possibility of producing zygotes carrying lethal genes. In *Saccharomycodes*, where this competition is eliminated by direct fusion of ascospores, a balanced lethal mechanism associated with mating types develops as was found by Winge and Laustsen ('39) in *Saccharomycodes ludwigii*. In *Saccharomyces* the strength of the mating-type alleles is adjusted to permit the haploid spores to germinate and produce small colonies before copulation occurs. The greater vigor of the legitimate diploid enables it to swamp out the haploid cells, and relatively few *Torulae* or *Zygosaccharomyces* are found in nature.

SUMMARY

Mating type alleles in *Saccharomyces cerevisiae* are unstable, and cultures capable of copulation become transformed in the laboratory into *Torulae* incapable of mating with each other or with tester strains. The instability of the mating type alleles results in the selection of mutants of diminished fertility during the period that the isolated haplophase is grown in pure culture.

Structures resembling copulation tubes are produced by exposure of yeasts to camphor. We have not been able to induce nuclear fusions by mating cultures in the presence of camphor.

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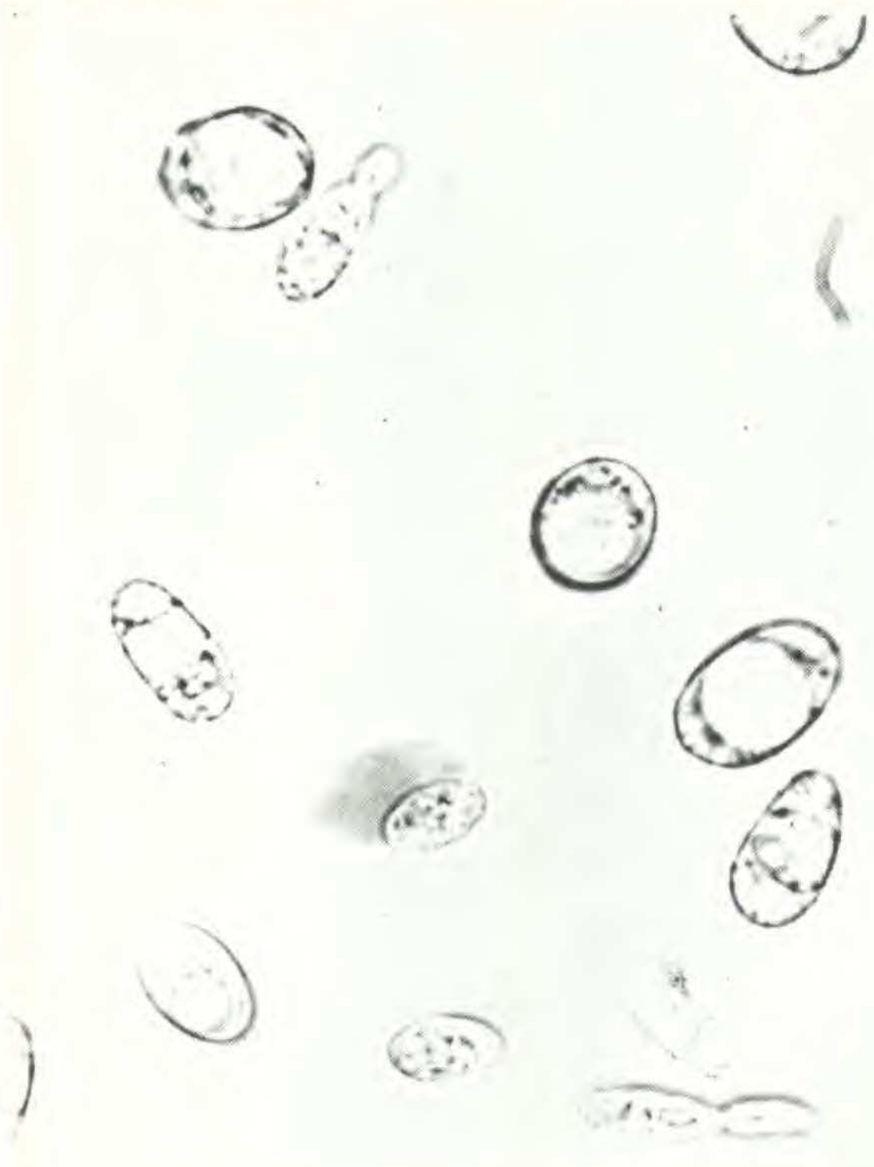
EXPLANATION OF PLATE

PLATE 7

Saccharomyces cerevisiae

The effect of camphor on diploid and haploid yeasts:

a. Diploid culture of the Lk strain grown on a nutrient agar plate without camphor.
b. Lk strain in same medium containing .3 per cent camphor.
c. Haploid culture of M3 strain grown without camphor.
d. M3 strain in medium containing .3 per cent camphor.



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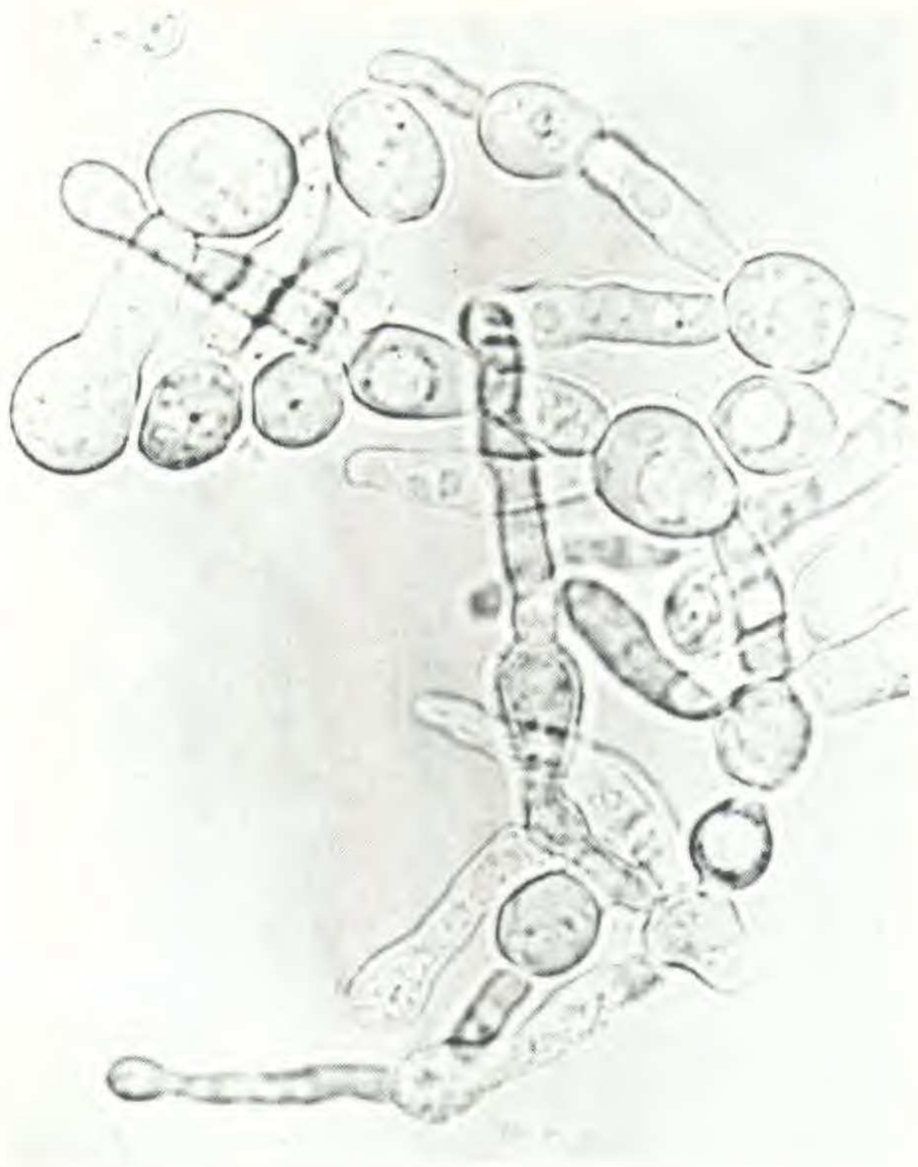

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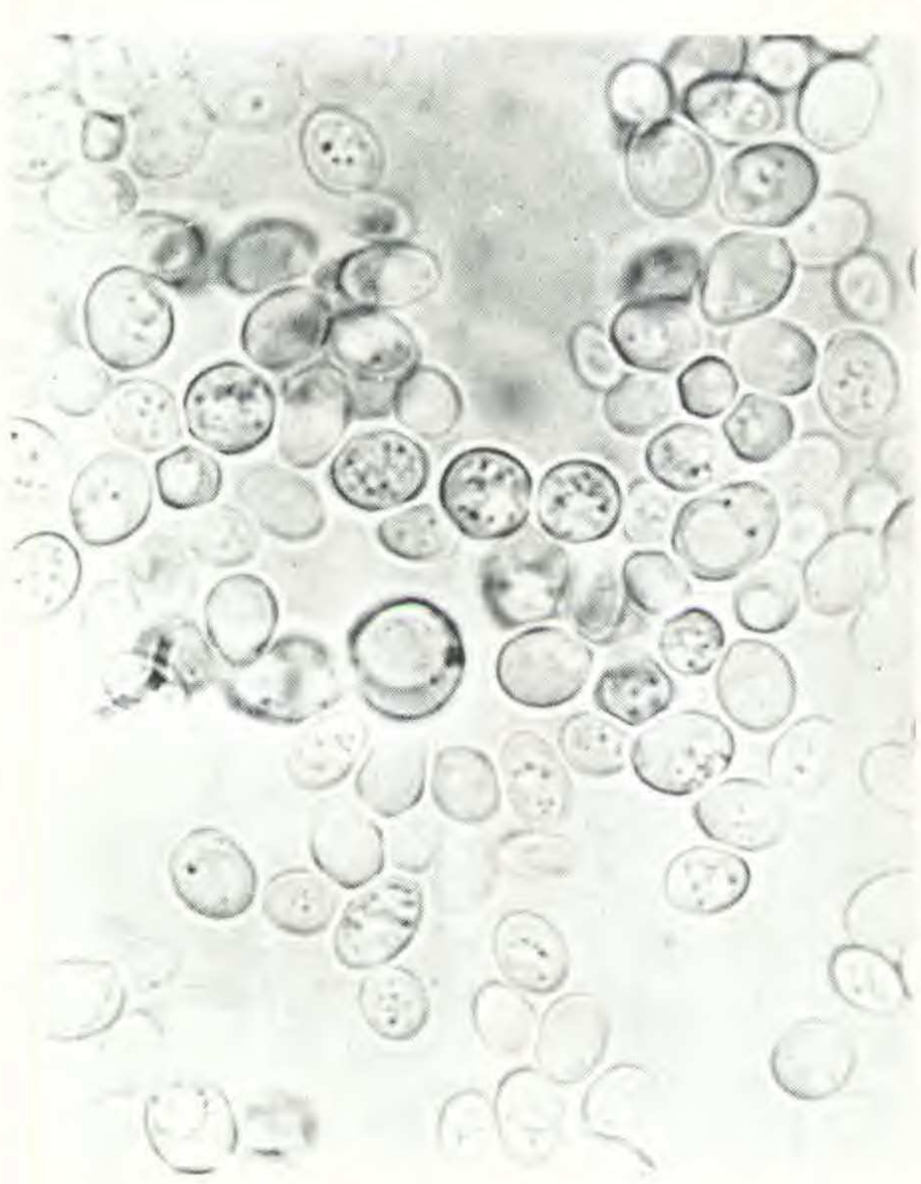

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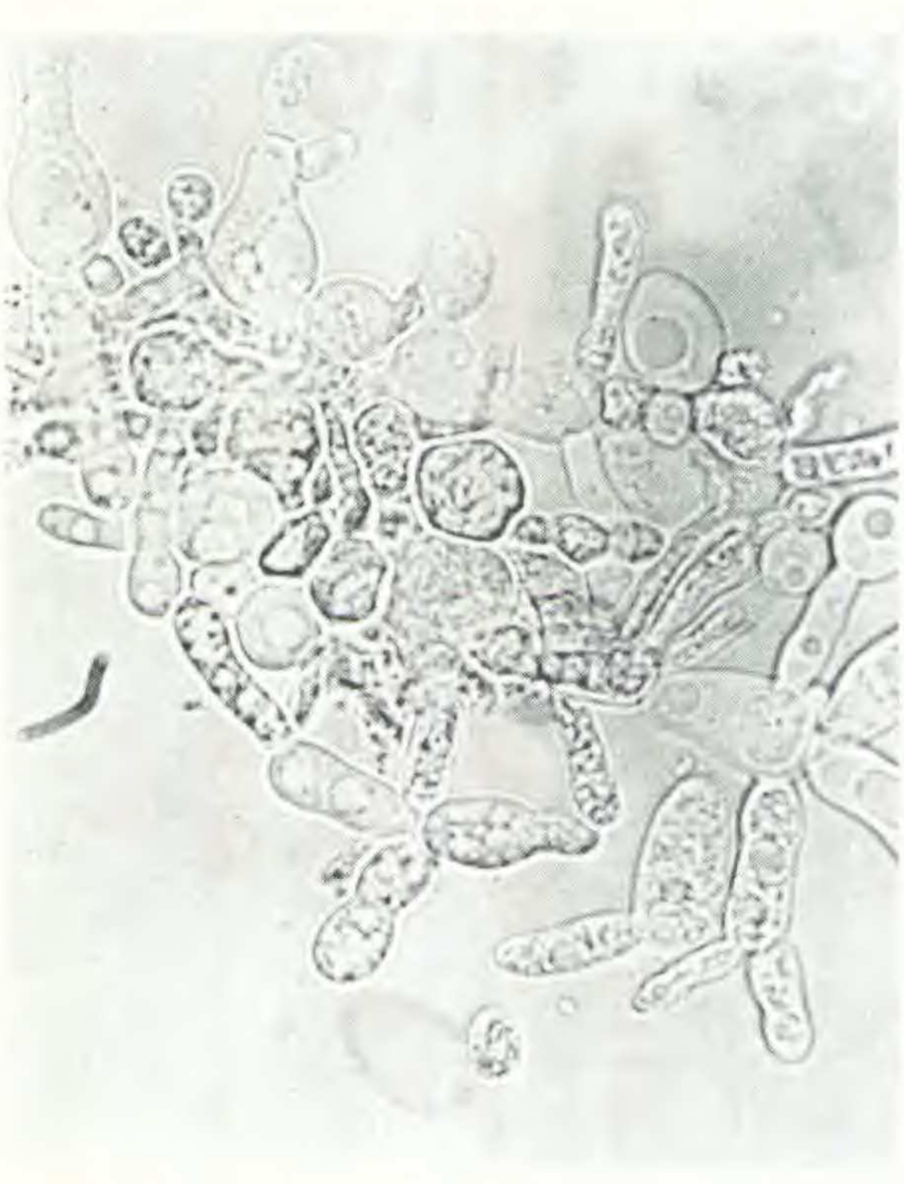

d

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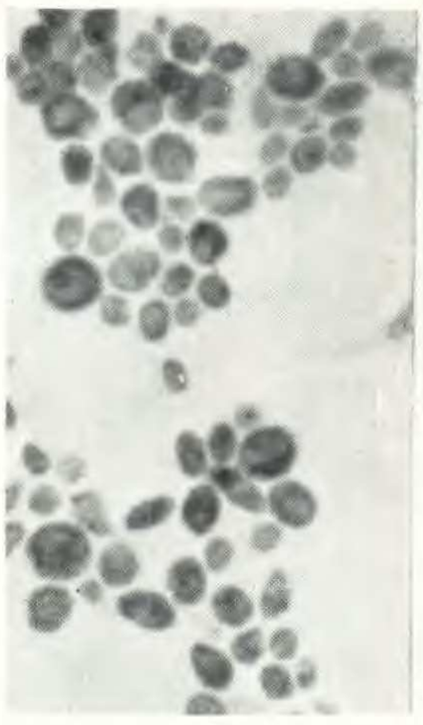
EXPLANATION OF PLATE

PLATE 8

Saccharomyces cerevisiae

Cells from the gypsum slants originating from the cultures and pairings shown in text-fig. 1.



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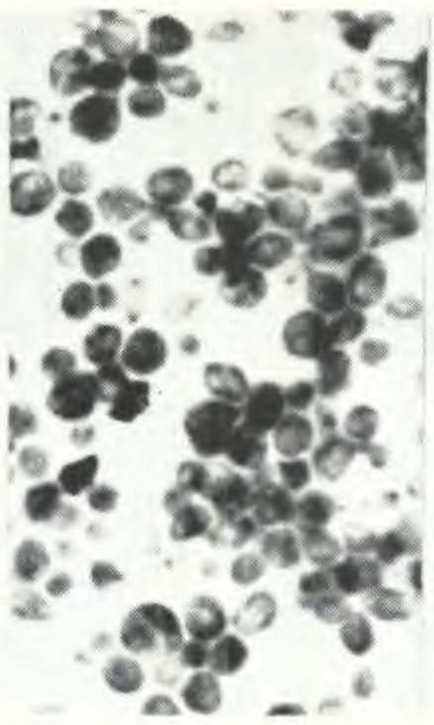

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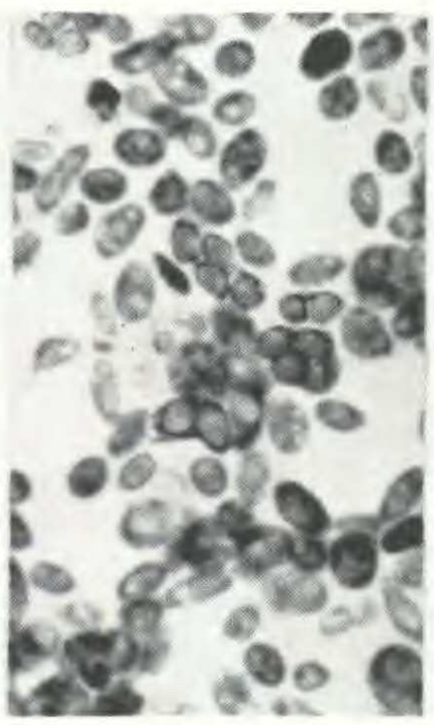

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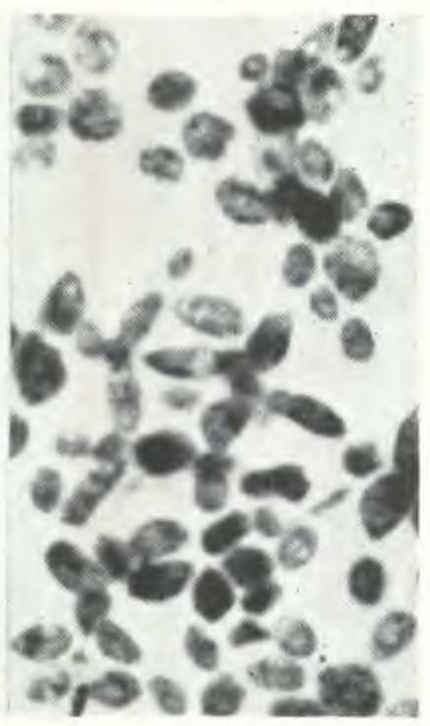

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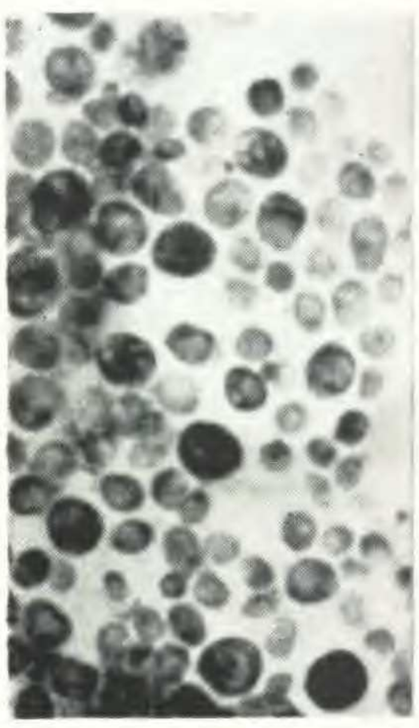

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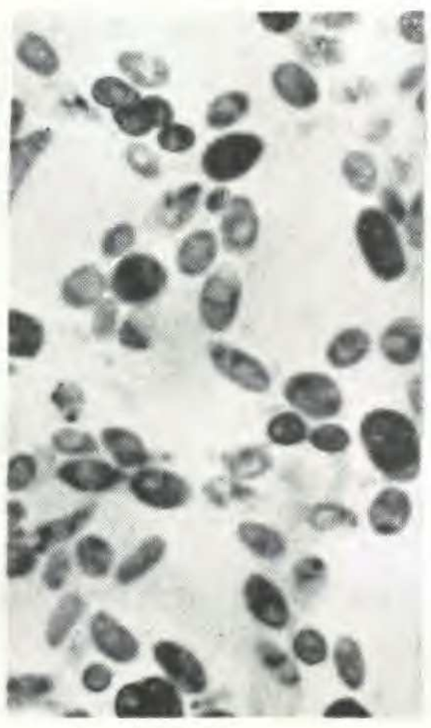

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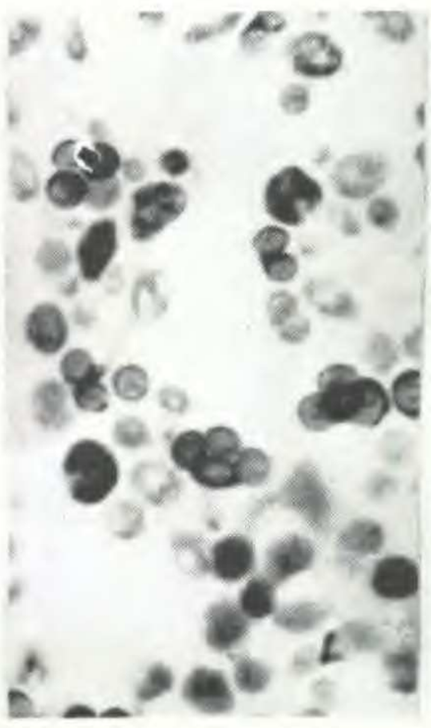

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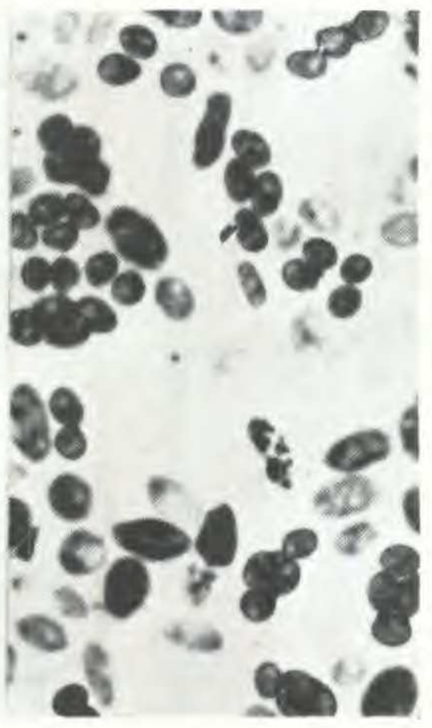

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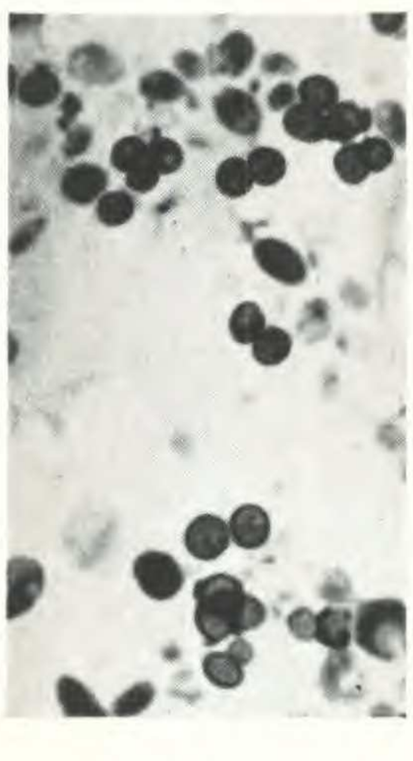

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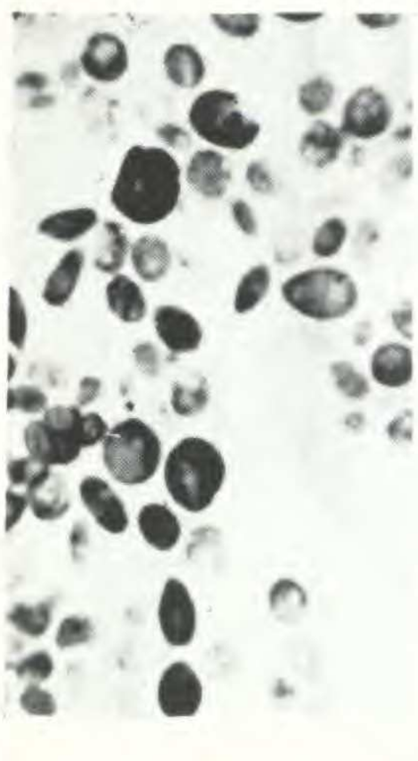

63 x 64

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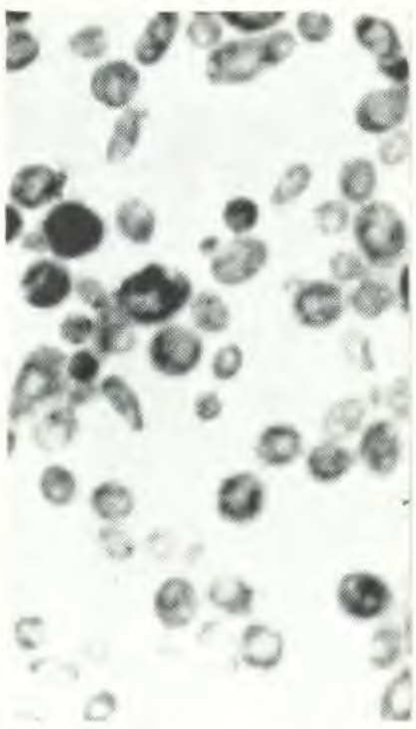
EXPLANATION OF PLATE

PLATE 9

Saccharomyces cerevisiae

Cells from the gypsum slants originating from the cultures and pairings shown in text-fig. 2.



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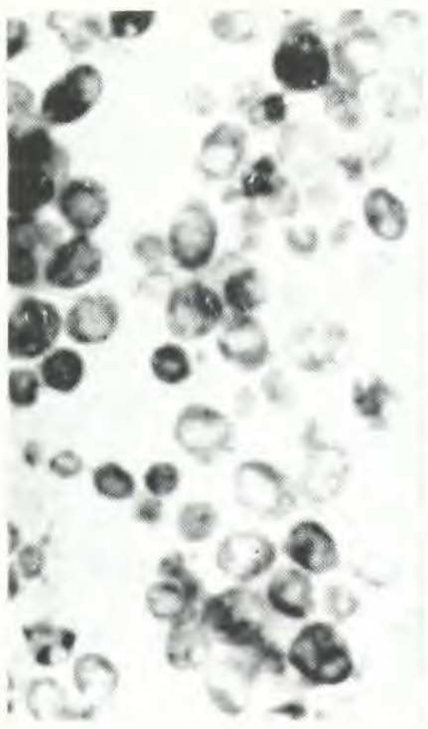

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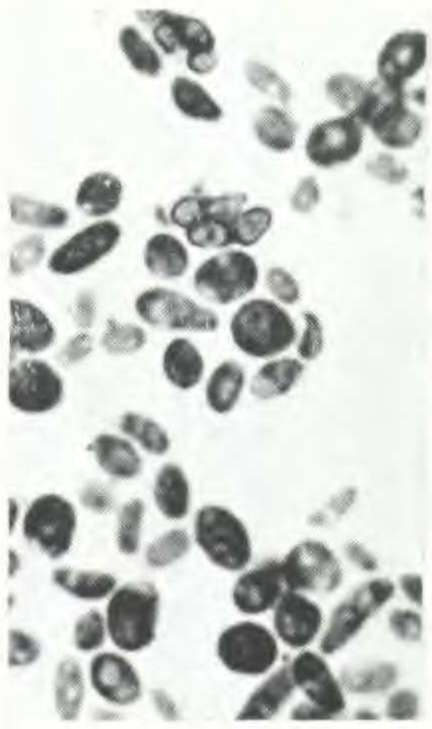
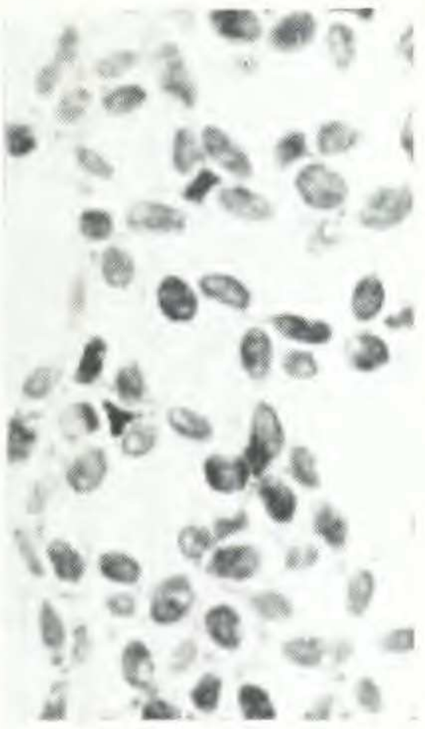


63



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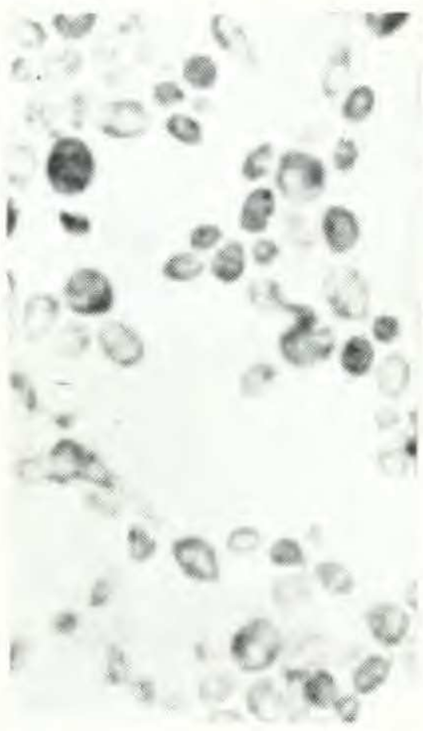

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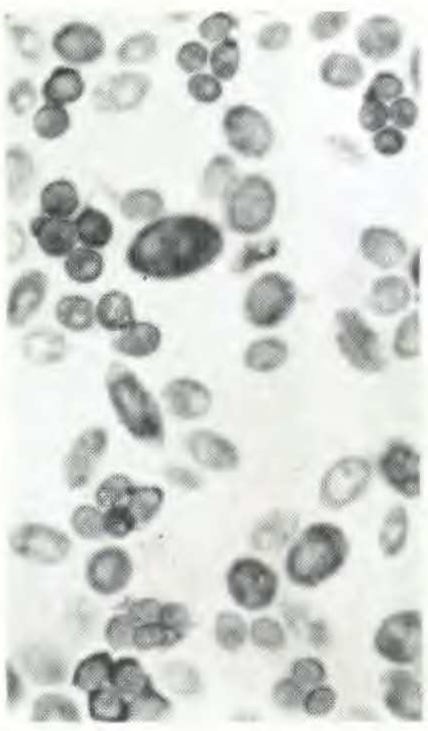

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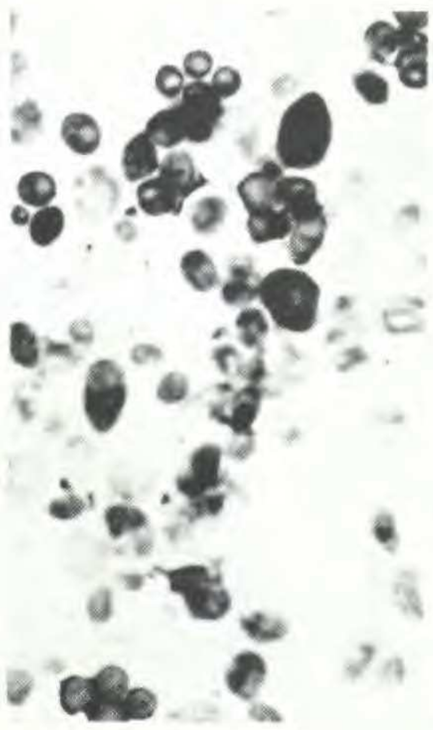

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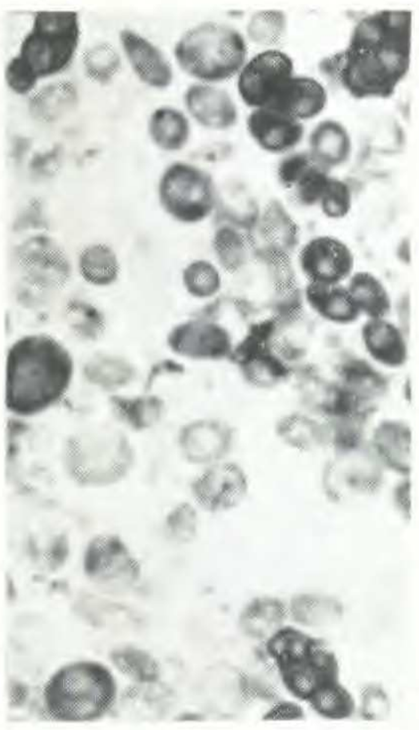

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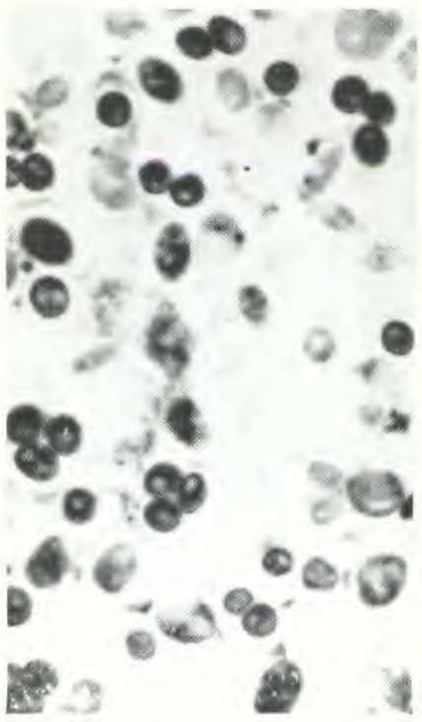

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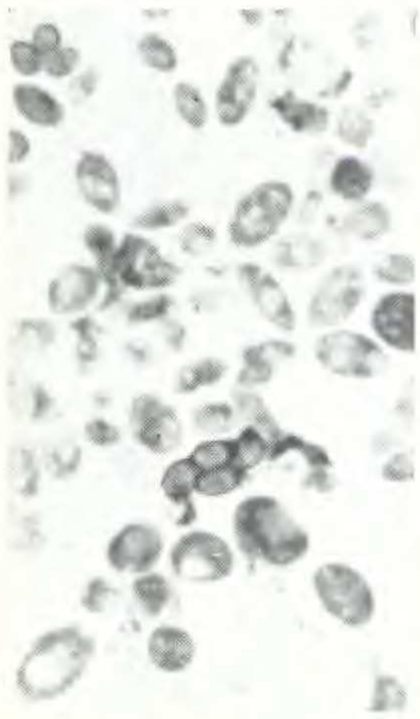

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